

SM (Streptomycin) ELISA Kit

Catalog No: E-FS-E031

96T/96T*3

Version Number: V1.1
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This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Test principle

This kit uses Competitive-ELISA as the method for the quantitative detection. It can detect Streptomycin (SM) in muscle, honey, etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody working solution, standard and other supplementary reagents. The microtiter plate in this kit has been pre-coated with coupled antigen. During the reaction, SM in the samples or standard competes with coupled antigen on the solid phase supporter for sites of anti-SM antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter plate well, and substrate reagent is added for color development. There is a negative correlation between the OD value of samples and the concentration of SM. The concentration of SM in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Reaction mode (Incubation time and temperature): 25±2°C; 30 min, 30 min, 15 min.

Detection limit: Muscle---4 ppb; Honey---2 ppb; Milk, Milk powder---5 ppb; Eggs---10 ppb; Cell culture supernatant---1 ppb.

Cross-reactivity: Streptomycin---100%; Dihydrostreptomycin---100%; Clarithromycin---6.3%; Gentamicin---2.5%.

Sample recovery rate: 85%±15%.

Kits components

Item	Specifications
ELISA Microtiter plate	96wells
Standard Liquid	1 mL each (ppb=ng/mL=ng/g) (0 ppb, 0.1 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb)
HRP Conjugate	11 mL
Antibody Working Solution	5.5 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
20×Concentrated Wash Buffer	40 mL
5×Reconstitution Buffer	50 mL
Plate Sealer	1 piece
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

Other materials required but not supplied

Instruments: Microplate reader, Printer, Homogenizer, Water bath, Vortex mixer, Centrifuge, Graduated pipette, Balance (sensitivity 0.01 g).

Micropipette: Single channel (20-200 µL, 100-1000 µL), Multichannel (30-300 µL).

Reagents: NaOH, Na₂HPO₄•12H₂O, NaH₂PO₄•2H₂O, N-hexane, 85% Phosphoric acid (H₃PO₄), Methanol, Acetic acid (AR, 99.5%).

Notes

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25±2°C.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. FOR RESEARCH USE ONLY. ELISA Microtiter plate should be covered by plate sealer. Avoid the kit to strong light.
5. **Each reagent is optimized for use in the E-FS-E031. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other E-FS-E031 with different lot numbers.**
6. Substrate Reagent should be abandoned if it turns blue color. When OD value of standard (concentration: 0) < 0.5 unit (A450nm < 0.5), it indicates the reagent be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.
8. As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.
9. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
10. **For mentioned sample fast and efficient extraction methods are included in the kit description. Please consult technical support for the applicability if other sample need to be tested.**
11. The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS, etc. can be used for quantitative confirmation.

Storage and expiry date

Store the kit at 2-8°C. Do not freeze any test kit components.

Return any unused microwells to their original foil bag and reseal them together with the desiccant provided and further store at 2-8°C. After opening, the kit is stable for up to 1 month.

Expiry date: expiration date is on the packing box.

Experimental preparation

Restore all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

Experimental apparatus should be clean, and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Solution preparation

Please prepare solution according to the number of samples. Don't use up all components in the kit at once!

Solution 1: **0.05 M PB Buffer** (*for muscle, milk, milk powder sample*)

Dissolve 12.9 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 2.175 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ to 1000 mL with deionized water, mix fully.

Solution 2: **0.04 M H_3PO_4 Solution** (*for honey sample*)

Dilute 1 mL of **85% H_3PO_4** to 360 mL with deionized water, mix fully.

Solution 3: **1 M NaOH Solution** (*for honey sample*)

Dissolve 4 g of **NaOH** to 100 mL with deionized water, mix fully.

Solution 4: **1% Acetic acid Solution** (*for egg sample*)

Dissolve 1 mL of **Acetic acid** to 100 mL with deionized water, mix fully.

Solution 5: **70% Methanol Solution** (*for egg sample*)

Dilute **Methanol** with deionized water, mix fully.

(Methanol (V): deionized water (V) =7:3).

Solution 6: **Reconstitution Buffer**

Dilute the **5×Reconstitution Buffer** with deionized water, mix fully.

(5×Reconstitution Buffer (V): Deionized water (V) = 1:4).

The Reconstitution buffer can be store at 4°C for a month.

Solution 7: **Wash Buffer**

Dilute the **20×Concentrated Wash Buffer** with deionized water, mix fully.

(20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19).

3. Sample pretreatment procedure

3.1 Pretreatment of muscle sample:

- (1) Remove fat from sample. Homogenize the representative sample with a homogenizer and mix fully.
- (2) Weigh 2 ± 0.05 g of homogenate sample without fat to centrifuge tube, add 8 mL of **0.05 M PB Buffer** (Solution 1). Oscillate for 5 min and incubate at 56°C water bath for 30 min.
- (3) Centrifuge at 4000 rpm for 5 min at room temperature.
- (4) Take 1 mL of the supernatant to another centrifuge tube, add 1 mL of **N-hexane**. Mix fully and centrifuge at 4000 rpm for 5 min at room temperature.
- (5) Discard the upper organic phase layer, take 50 μ L of the lower liquid to another centrifuge tube. Add 450 μ L of **Reconstitution Buffer** (Solution 6), mix fully for 30 s.
- (6) Take 50 μ L for analysis.

Note: Sample dilution factor: 40 detection limit: 4 ppb.

3.2 Pretreatment of honey sample:

- (1) Weigh 2 ± 0.05 g of honey to centrifuge tube, add 4 mL of **0.04 M H₃PO₄ Solution** (Solution 2), oscillate until dissolved fully. Centrifuge at 4000 rpm for 5 min at room temperature.
- (2) Take all of supernatant to another centrifuge tube. And add 450 μ L of **1 M NaOH Solution** (Solution 3) to adjust the pH to 7-9. Centrifuge at 4000 rpm for 5 min at room temperature.
- (3) Take 50 μ L of supernatant and add 450 μ L of **Reconstitution Buffer** (Solution 6) to another centrifuge tube, mix fully for 30 s.
- (4) Take 50 μ L for analysis.

Note: Sample dilution factor: 20 detection limit: 2 ppb.

3.3 Pretreatment of milk and milk powder sample:

- (1) Weigh 2 ± 0.05 g of sample to centrifuge tube, add 8 mL of **0.05 M PB Buffer** (Solution 1), oscillate for 5 min, incubate at 56°C water bath for 30 min.
- (2) Centrifuge at 4000 rpm for 10 min at room temperature.
- (3) Discard the upper fat layer and take 50 μ L of clear liquid of the middle layer to another centrifuge tube. Add 450 μ L of **Reconstitution Buffer** (Solution 6), mix fully for 30 s.
- (4) Take 50 μ L for analysis.

Note: Sample dilution factor: 50 detection limit: 5 ppb.

3.4 Pretreatment of cell culture supernatant sample:

- (1) Take 0.1 mL of cell culture supernatant into 1.5 mL centrifuge tube, add 0.9 mL **Reconstitution Buffer** (Solution 6), vortex for 1 min.
- (2) Take 50 μ L for analysis.

Note: Sample dilution factor: 10 detection limit: 1 ppb.

3.5 Pretreatment of eggs sample:

- (1) Remove shells of eggs, and homogenize white and yolk of the egg with homogenizer.
- (2) Weigh 1 ± 0.05 g of homogenate sample to 50 mL centrifuge tube, add 2 mL of **1% Acetic acid Solution** (Solution 4), oscillate for 2 min.
- (3) And add 7 mL of **70% Methanol Solution** (Solution 5), oscillate for 2 min, Centrifuge at 4000 rpm for 10 min at room temperature.
- (4) Take 0.1 mL of supernatant to 1.5 mL centrifuge tube. And add 0.9 mL of **Reconstitution Buffer** (Solution 6), mix fully for 30 s.
- (5) Take 50 μ L for analysis.

Note: Sample dilution factor: 100

detection limit: 10 ppb.

Assay procedure

Restore all reagents and samples to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8°C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. **Standard and Samples need test in duplicate.**
2. **Add Sample:** add 50 μ L of **Standard** or **Sample** per well, then add 50 μ L **Antibody Working Solution**, cover the plate with plate sealer. Oscillate for 5 s gently to mix thoroughly, incubate at $25\pm 2^\circ\text{C}$ for 30 min with shading light.
3. **Wash:** uncover the sealer carefully, remove the liquid in each well. Immediately add 350 μ L of **Wash Buffer** (Solution 7) to each well and wash. Repeat wash procedure for 5 times, 30 s intervals/time. Invert the plate and pat it against thick clean with absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP Conjugate:** add 100 μ L **HRP Conjugate** to each well, incubate at $25\pm 2^\circ\text{C}$ for 30 min with shading light.
5. **Wash:** Repeat step 3.
6. **Color Development:** add 50 μ L of **Substrate Reagent A** to each well, and then add 50 μ L of **Substrate Reagent B**. Gently oscillate for 5 s to mix thoroughly. Incubate at $25\pm 2^\circ\text{C}$ for 15 min with shading light. (The reaction time can be extended according to the actual color change).
7. **Stop Reaction:** add 50 μ L of **Stop Solution** to each well, oscillate gently and mix thoroughly.
8. **OD Measurement:** determine the optical density (OD value) of each well at 450 nm (reference wavelength 630 nm) with a microplate reader. This step should be finished in 10 min after stop reaction.

Result analysis

1. Absorbance% = $A/A_0 \times 100\%$

A: Average absorbance of standard solution or sample

A_0 : Average absorbance of 0 ppb Standard solution

2. Drawing and calculation of standard curve

Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value to standard curve to get corresponding concentration. **If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.** For this kit, it is more convenient to use professional analysis form for accurate and fast analysis on a large number of samples.

Streptomycin (E-FS-E031) Standard Curve

